

Characterization of domains regulating antagonist-mediated down-regulation of 5-HT₇ serotonin receptors

The human 5-HT₇ serotonin receptor is a G-protein-coupled receptor that activates adenylyl cyclase constitutively and upon agonist-activation. In previous studies, some inverse agonists induce both homo- and heterologous desensitization, similar to agonist-stimulation. In addition, some inverse agonists induce receptor internalization, whereas a subset of these targeted 5-HT₇ receptors for lysosomal degradation. These results demonstrated that various ligands differentially activated regulatory processes governing receptor desensitization, internalization and degradation in addition to signal transduction, providing support for the concept of functional selectivity at the 5-HT₇ receptor; where different ligands stabilize different receptor conformations leading to differential effects. Interestingly, the important atypical antipsychotics olanzapine and clozapine blocked G-protein activation, but induced both internalization and degradation of 5-HT_{7(b)} receptors. Furthermore, 5-HT_{7(b)} receptors C-terminally fused to YFP did not undergo this degradation, indicating that key regulatory proteins bind to the C-terminal tail of 5-HT₇ receptors

In this study, the two important and relatively novel YXXΦ motifs were identified in the C-terminus of the 5-HT₇ receptor as potential sites involved in receptor internalization and recruitment of lysosomal sorting proteins such as sorting nexin 1 (SNX1) and GPCR-associated sorting protein (GASP). Mutation in either or both YXXΦ-motifs inhibited clozapine-mediated degradation of 5-HT_{7(b)} receptors. Using radioligand binding and adenylyl cyclase assays, the YXXΦ-mutant receptors showed no change in ligand-affinity, but displayed constitutive activity and an increase in basal AC activity. Therefore, YXXΦ-mutated motif(s) may induce a conformational change in the receptor C-tail which could lead to a different capacity of the C-tail to bind and activate G-protein in addition to regulating receptor internalization and trafficking. Furthermore, mutating both YXXΦ motifs block 5-HT-stimulated AC activity, even though the receptor is present on the plasma membrane and displays functional ligand-binding and constitutive AC activity.

In addition, 5-HT_{7(b)} receptors are constitutively degraded in both lysosomes and proteasomes, but a higher proportions of receptors are constitutively targeted to lysosomes. Incubation with clozapine, olanzapine or SB269970 sort receptors to both lysosomes and proteasomes. This study shows that YXXΦ-motifs are involved in internalization and lysosomal sorting of 5-HT_{7(b)} receptors.